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THE FUNCTION OF THE CORPUS ALLATUM IN MUSCOID DIPTERA

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Introduction

Since the original work of Wigglesworth (1926) it has become increasingly obvious that the corpora allata may exert an influence on the reproductive processes of insects (see Scharrer, 1941, for review). Although Thomsen (1940) concludes that in the adult muscid flies the single median corpus allatum controls the ripening of the ovaries, it is not yet established whether this action is a direct one, and whether there is a true sex hormone produced by the corpus allatum. The fact that Thomsen (l.c.) was able to induce hypertrophy of the corpus allatum by ovariectomy suggests a direct hormonal action, but cannot be taken as proof. In an attempt to throw further light on this problem the experiments to be described were performed. They are concerned with the role of the ring gland in the adult fly, and while the problem is as yet unsettled, considerable information has been gained and a tentative conclusion may be reached. The significance of the work lies in the fact that it bears finally on a major biological problem—the mode of action of hormones on cells and tissues.

Materials and Methods

Two species of Muscidae have been employed. A pure strain of *Lucilia sericata* Meig. maintained by mass inbreeding for over 230 generations was used in all earlier work. Search for a more robust fly resulted in the use of a stock of *Sarcophaga securifera* Villeneuve obtained from eggs deposited on meat, in the fall of 1941 in St. Louis, Missouri. Since that time the stock has been maintained by mass inbreeding in the laboratory.

The experiments have consisted mainly of extirpations and implantations of adult organs. Operations were performed under a magnification of 30 diameters and the essential instruments were No. 12 hard steel needles, appropriately sharpened on a hard Arkansas oilstone, and fine iridectomy forceps. Flies were etherized and held in "Permoplast" with cross pins. The corpus allatum may be removed through the neck region if the head of the fly is bent forward; gonads are removed by making a long transverse incision in the intersegmental membrane between the 4th and 5th abdominal segments. With practice the mortality can be reduced to very low figures for both operations, but I have never succeeded in performing both extirpations on a single fly, even when a day elapsed between the experiments.

In the experiments involving the transplantation of the ring gland, portions of the oesophagus invariably had to be included with the transplant, for the ring gland alone was too small to be moved without injury. The site of implantation was under the dorsal abdominal wall between the 2nd and 3rd scuta. In later experiments, in an effort to induce innervation of the transplant, attempts were made to place the ring gland near the brain, which was slightly injured in order to stimulate the growth of nerves to the implant. These attempts were unsuccessful.

A total of 73 successful operations were performed on Lucilia and 102 on Sarcophaga.

At the conclusion of all experiments the flies were fixed by injection with alcoholic Bouin's, and 10 micron paraffin sections were stained either in Mallory's triple stain or by Bodian's protargol technique. Results with both species of flies were similar, unless the contrary is stated.

Description of the Normal Histology

The histological effects of extirpation of the ring gland have not previously been described. In order that they may be more easily followed, a brief description of the normal histology is necessary.

A. *The Ring Gland.* The ring gland of *Lucilia* has been shown (Day, 1942) to be composed of a single median corpus allatum fused with the corpora cardiaca and the hypocerebral ganglion. The situation is similar in Calliphora (Thomsen, 1941) and in Sarcophaga (Figures 3, 4, and 5). Thomsen (1941) refers briefly to the larger size of the corpus allatum in mature flies compared with newly emerged flies (compare her Figures 3 and 4). The changes occurring during the first seven days after emergence of adult *Lucilia sericata* and from emergence to 20 days of age of Sarcophaga, have been carefully followed. The most striking changes occur in the first five days and thereafter there is not much alteration

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Plate I

Corpora allata and fat body of *Lucilia sericata* and *Sarcophaga securifera* fixed in alcoholic Bouin, Bodian protargol method. Photomicrographs, magnification 400 diameters.

1. Corpus allatum of normal female *L. sericata*, transverse section. Note larger nuclei on periphery of the gland, and below a little striated muscle from the dorsal vessel. Cell walls are not easily seen but are present.

2. The same of a female castrated seven days. Note the hypertrophy of the cells and nuclei. The gland approximately 50 per cent greater in diameter than the control even though there are fewer cells in the section. The hypertrophy of the nuclei is particularly striking and is shown by the larger peripheral ones as well as the central ones. Cell walls are clearly seen.

3. Corpus allatum of *S. securifera* in which recurrent nerve was cut seven days earlier. Note nerve fibers ramifying between cells. In comparison with Figures 4 and 5 note the hypertrophy of cells and nuclei.

4. Corpus allatum of male castrate *S. securifera*.

5. Corpus allatum of female castrate *S. securifera*. Note that there is no hypertrophy in either this gland or that in Figure 4 comparable with that found in Lucilia.

6. Fat body of female *Sarcophaga securifera* showing darkly stained oenocytes. Tissues are essentially normal. Compare with Figure 7.

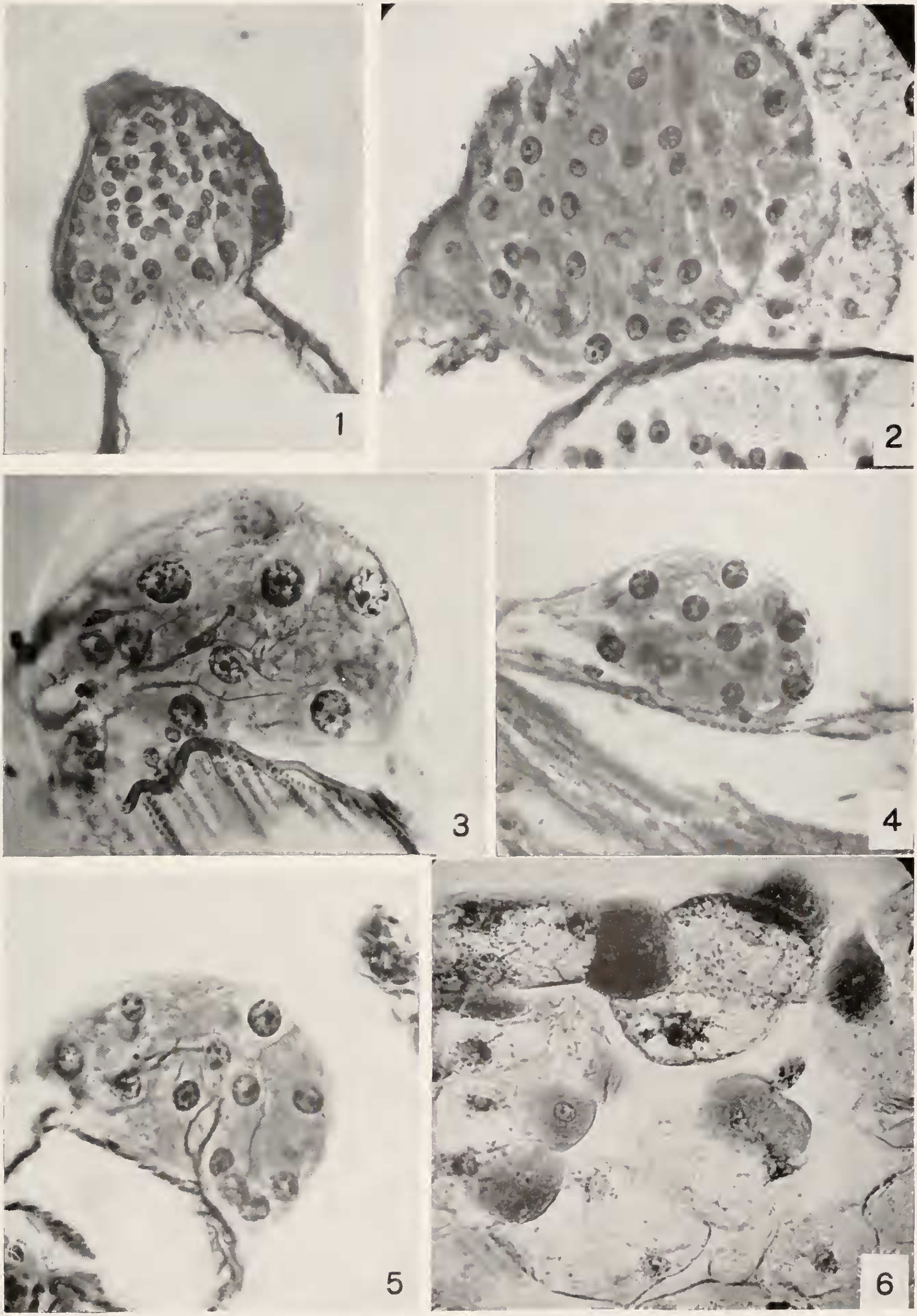

PLATE I

in the cytology of the gland. Differences between the sexes are insignificant. In Sarcophaga all the fuchsinophilic droplets in the corpus cardiacum cells have disappeared at the time of emergence, but persist for about three days in Lucilia (Day, 1942). In Lucilia larger nuclei seem to occur on the periphery of the gland, but this is not so marked in Sarcophaga. In both there is a thin sheath, which has small flattened nuclei, surrounding the gland. The innervation of the gland is well shown in Bodian preparations and, as in other insects, is very profuse (see especially Figures 3 and 4). Nerves from the hypocerebral ganglion run dorsally in the lateral walls of the aorta and ramify between almost every cell of the corpus allatum. It is almost certain that fine nerves penetrate cells and end near the nucleus in small terminal swellings. Cell membranes are not clearly seen in the illustrations, but the gland is not syncytial. It is well tracheated, but no specializations for transferring secretory products to the aorta are found.

B. *The Fat Body.* As will be shown later the fat body undergoes striking changes after allatectomy. Its normal histology is therefore discussed here and considerable attention has been paid to this tissue in all experimental animals. Teunissen (1937) and Pérez (1918) have described the changes in the fat body of flies during the pupal period, but no adequate description is to be found of subsequent changes. Evans (1935) has described the fat body of adult Lucilia and Roubaud (1932) has described chemical changes in that of *Culex pipiens.* In Sarcophaga I have found that larval fat body cells persist in normal flies for three days after emergence (Figure 7). Their fuchsinophilic contents gradually diminish until the cytoplasm becomes clear and reduced, though the large nucleus still makes it easy to distinguish the cells. The adult fat body cells are at first small but during the first three days of adult life they gradually increase in size. It should be noted that fat body cells differ in various parts of the body. This discussion is limited to the fat body of the lateral body wall in the segments containing the gonads. No substantial difference was observed between the sexes.

Young cells show most frequently four nuclei, but may possess more. A count of the number of nuclei in fat body cells of Sarcophaga gave the following results: Two nuclei were found in 15 per cent of the cells, four in 50 per cent, six in 5 per cent and eight in 30 per cent. The counts were made from acetic orcein spreads from which counts may be made easily and accurately, while this is not possible from sections. No significant change was found between young and old flies, nor is there a difference between the sexes. There is, however, a marked difference depending on where the fat body is located. Cells near the heart are smaller and have fewer nuclei than those located on the lateral body wall. When the fat body is abundant the cells composing it are closely appressed and their exact connections are impossible to determine. In living spread preparations, especially of older flies in which the fat body is less abundant, it is found that the fat body cells are arranged in cords which branch and in which the oenocytes lie in between almost every two cells. In young cells the nuclei are spherical and possess a single, usually slightly eccentric nucleolus. The cytoplasm is aggregated around the periphery of the cell. Within 24 hours after emergence the cells have increased considerably in size, the nuclei have become more nearly centrally located and the nuclear membrane has become slightly irregular in outline and their nucleoli have enlarged considerably. The cytoplasm is uniform, but is lightly aggregated around the nuclei. If the flies continue on a carbohydrate diet no further change occurs. A protein meal, however, changes the appearance of the cytoplasm considerably. It becomes more abundant and thick strands run from the cell wall to the nuclei. The nuclei frequently regain their spherical shape.

In Lucilia, the changes undergone by the fat body are essentially similar to those just described (see Evans, 1935).

C. *The Oenocytes.* Intimately associated with the fat body are the oenocytes. Snodgrass (1935, p. 411) made the undocumented statement that "oenocytes are not known to occur in adult Diptera." However, oenocytes in adult Diptera have been described by Pérez (1910) in Calliphora and by Evans (1935) in Lucilia.

In the newly-emerged Lucilia they are not striking, but with the increase in size of the fat body cells the oenocytes become more conspicuous. They are characterized by uniform, basophilic cytoplasm. Many of the cells, which are uniformly scattered among the abdominal fat body cells, are uninucleate, but about 50 per cent are binucleate. More rarely three or four nuclei are found. Nuclear size in Lucilia oenocytes with a single nucleus averages about six microns, but is less when there are more nuclei per cell. It was early noticed that there was a marked and constant difference between the oenocytes of the two sexes of Lucilia. While those of the male were large and well filled with cytoplasm so that the cell boundaries were convex, those of the females were less conspicuous and had concave cell boundaries. This difference could be clearly seen in the living fat body if the fly was injected with a solution of methylene blue which stains oenocytes specifically. When a similar condition was found in such widely separated flies as *Melophagus ovinus* L. and *Culex pipiens* L., it was thought that the situation might be general in the Diptera. It was so striking that it seemed surprising that no record of this could be found in the literature. It was therefore unexpected to find that *Sarcophaga securifera* did not conform in this respect, the oenocytes being if anything more conspicuous in the female than in the male, although there was little difference between the sexes. The explanation for this is quite unknown but is obviously significant in the explanation of the sexual differences, which must lie eventually in a knowledge of the function of the oenocytes. Without diverging unnecessarily it seems that the majority of evidence points to their functioning as organs of intermediary metabolism (see Wigglesworth, 1939, p. 244). The validity of the interpretation of the following results rests in part on the very plausible assumption that this is at least one of their functions.

In normal flies the oenocytes are very constant, and no cytological evidence of secretory changes can be seen in the adult, though there are some indications that they show an inverse size relationship with the cells of the fat body, and vary slightly with the nutritional state of the insect. As will be shown below, they undergo marked changes upon extirpation of the ring gland.

D. *The Ovaries.* For purposes of subsequent descriptions it is necessary to review briefly the growth of the eggs in the ovary of Lucilia. The relation between nutrition and egg production of muscids has been discussed by Glaser (1923) and Mackerras (1933), and the development of the ovaries of Anopheles by Nicholson (1921). Nicholson divided the growth of the oöcyte into two stages. The first of these represents the growth up to a resting stage in which the oöcytes remain until the insect has taken a protein meal (see Trager, 1941,

p. 23). Histologically such oöcytes are easily distinguished, for no yolk has yet been laid down in them. In Sarcophaga the follicle attains a maximum diameter of about 160 microns. Immediately after feeding on meat, yolk is deposited and the oöcyte increases in size to about three times the size reached in stage I. The nurse cells undergo little change. Once yolk deposition has begun the follicles increase rapidly in size. The follicular cells increase in number but decrease in size. They change from cuboidal towards squamous cells. Later the chorion is laid down.

The Effects of Extirpation of the Ring Gland

As mentioned above, extirpation of the ring gland of adult flies can be performed with comparative ease. It is, however, not possible to determine without histological examination whether both corpora allata and cardiaca are removed. Thomsen records that cardiacectomy was avoided in her operations. Most frequently in this work the corpora cardiaca were removed with the corpus allatum. After allatectomy alone, however, the effects were indistinguishable from complete extirpation of the ring gland. About 80 per cent of the flies operated upon in this way live apparently normally and show no external signs of their operation. Mortality is about 10 per cent and the remaining 10 per cent show the water balance upset described below.

Among the 80 per cent of operated flies that survived, normal mating reactions have been noted. The majority of flies (37 Lucilia, nine Sarcophaga) have been operated upon when 24 hours old, and subsequently fed sugar and water, and fixed after seven days. Thirteen cases in Lucilia were allowed to live to 21 days after the operation and three cases in the Sarcophaga series were fed meat for four days. However, eggs were never developed, though in one case a little yolk was found in an oöcyte slightly enlarged beyond stage I.

In spite of their normal behavior, operated flies present an unmistakable histological picture differing from the controls in regard to the fat body, ovary,

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Plate II

Fat body of *Lucilia sericata* and *Sarcophaga securifera*. Fixed with alcoholic Bouin's. Photomicrographs, magnification 400 diameters, except Figure 7 which is $\times 170$.

7. Fat body of normal male *S. securifera* three days of age. Note the larval fatbody cell whose fuchsinophil droplets have almost disappeared. Compare the oenocytes with those of the female (Figure 6). Note that the magnification is only 170 diameters.

8. Fat body of female *L. sericata* in which recurrent nerve had been cut seven days. The reduced and pycnotic oenocytes, pycnotic small dark nuclei of the fat body are characteristic of flies after extirpation of the ring gland.

9. Fat body of male *S. securifera* allatectomized seven days. Note same effects as seen in Figure 8. The separation of the cytoplasm from the cell wall is characteristic.

10. Fat body of female *S. securifera* allatectomized for seven days when female was six days old. Note less marked effect than in Figure 9, though the effects of the operation can be seen in comparison with Figure 12.

11. Fat body of female *S. securifera* seven days after the extirpation of the ring gland with ring gland from female castrated seven days implanted for 46 hours. In comparison with Figure 9 the cytoplasm and nuclei are seen to have undergone conspicuous changes. Oenocytes, however, are unaffected.

12. As Figure 11, except that the transplanted ring gland was from a normal seven-day old female. Note that the fat body is almost normal, but the oenocytes still show the effects of allatectomy.

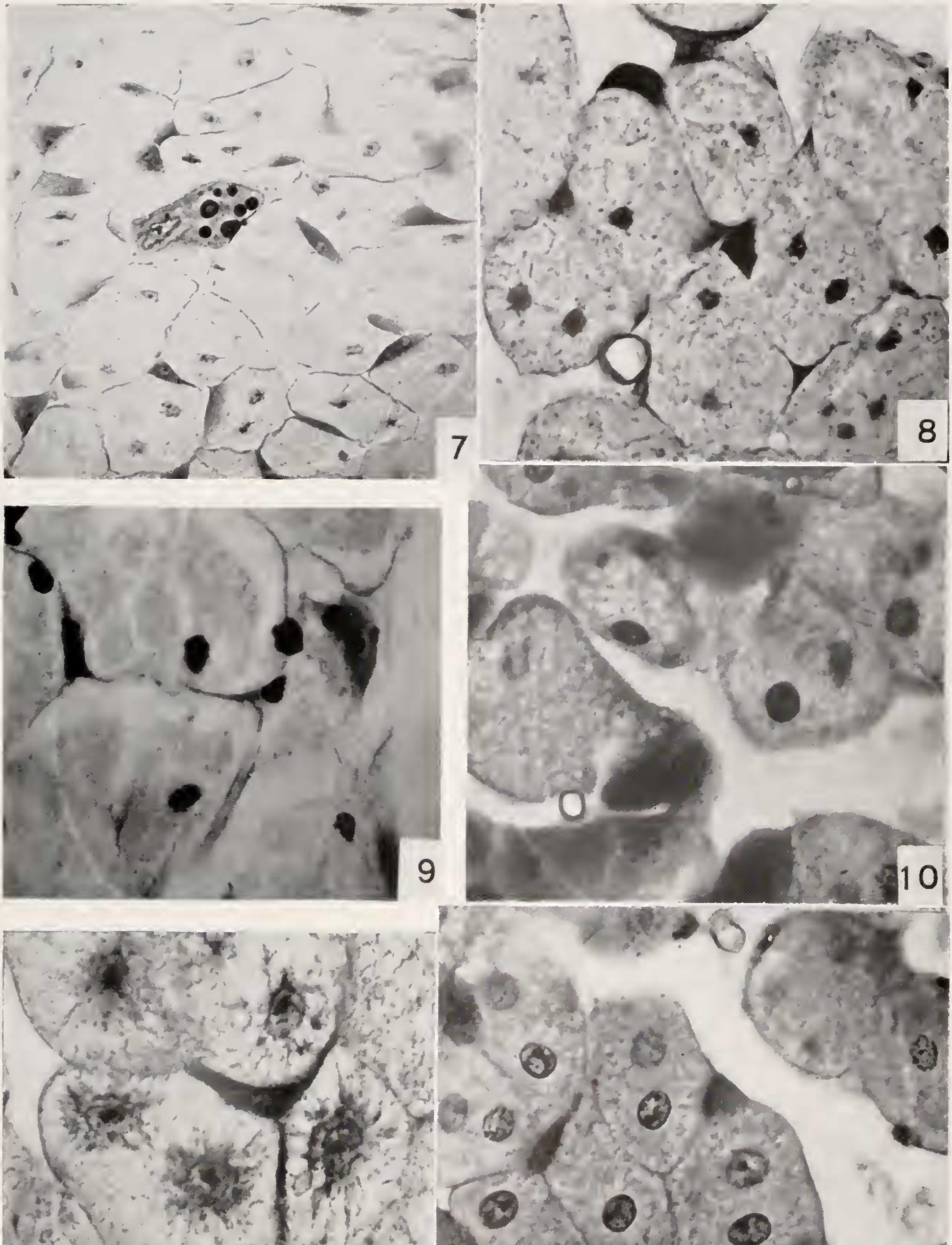

PLATE II

and oenocytes. No effects could be seen on testes, on the nervous tissue, including the neurosecretory cells of the brain (Day, 1940), alimentary canal, Malpighian tubules, or on the accessory glands of the reproductive system of either the male or the female.

A. The effects on the fat body become visible within three days after the operation, but little change occurs from then up to 21 days. If the operation is performed on a fly within two days after emergence, the cells of the larval fat body never completely disappear, as they do in the normal fly. Even after the acidophilic cytoplasmic inclusions (see Figure 7) have disappeared, the larval cells can easily be recognized by the large size of the nuclei. Thus one of the effects of extirpation of the ring gland is to inhibit the normal maturation of certain tissues. The extent of the change in the adult fat body may be seen by comparing Figures 7 and 9. Many of the nuclei of the fat body cells in the operated flies become pycnotic, have crenulated borders, and are greatly decreased in size. A still more striking change occurs in the cytoplasm which appears much more sparse than in the controls. No specific stains have been used to attempt to learn exactly what components have disappeared from the cytoplasm but the general appearance is that of a cell whose reserves have been in large part utilized. It would be desirable to determine in what way these changes correlate with alterations in physiological function. The results suggest a comparison with the findings of Pfeiffer (1941) on Melanoplus that allatectomy results in a greatly increased fat content. While it will be seen from Figures 7 and 9 that there is an increase in fat body cell size following extirpation of the ring gland, an increase in fat does not appear to occur in female Lucilia or Sarcophaga after this operation although the vacuoles observed in the fat body cells may be left by dissolved fat.

If the operation is performed on a fly six days of age, the fat body does not regress, but the effect is still seen upon the oenocytes (compare Figure 10).

B. Even more striking changes occur in the oenocytes following extirpation of the ring gland. Such changes have not been previously reported for any insect. The effects are not quite comparable in Lucilia and in Sarcophaga. In males of the former species the large oenocytes of the male are most markedly affected, being much reduced in size. Their nuclei become pycnotic and their cytoplasm changes from an homogeneous basophilic to a strong acidophilic reaction. Comparable but less marked changes occur in the female Lucilia. In Sarcophaga the cytoplasm does not become so markedly reduced, but the cell boundaries of the oenocytes almost invariably become indistinct (Figure 9). As in Lucilia the nuclei show varying degrees of pycnosis and in both sexes the cells are greatly reduced in size. Feeding protein in addition to carbohydrate has little effect on these oenocytes.

C. Extirpation of the ring gland produces an effect upon the ovaries. If the operation is performed on young females fed only sugar, development beyond Stage 1 is never found. The histology of the eggs usually remains normal. However, in one case in which a female Sarcophaga was allatectomized when two days old, considerable regression of the ovaries was found. Practically no oöcytes were present and the ovaries consisted of a mass of knotted tracheae and a few small connective tissue and muscle cells.

If the operation is performed on a female in which the ovaries are well developed, degeneration of certain oöcytes occurs. Wigglesworth (1936) reported a similar situation in Rhodnius. The eggs on the periphery are affected first and the changes are exactly comparable to those seen in old females inhibited from ovipositing. Apparently extirpation of the ring gland hastens this process and causes its occurrence in a greater number of oöcytes than normal. The first indication of such degenerative changes is seen in an increase in size of the follicular cells. These large cells then begin to phagocytize the acidophilic yolk granules, and persist for a while with darkly staining masses in their cytoplasm. Eventually they digest this material and the yolk decreases in quantity until in the last stages that have been observed, the oöcyte contains practically no yolk.

Previous authors have described changes in the secretions of the oviduct as a result of allatectomy. These were not observed in Lucilia or Sarcophaga. Similar changes in the fat body and oenocytes occur in males as well as females, but the testes and accessory glands are unaffected.

D. As mentioned above, the effects of removing the corpus allatum just described are sometimes modified. In about 10 per cent of the operations the flies exhibit a marked distension of the abdomen, sometimes within five hours, but more usually about 12 hours after the operation. Complete serial sections of these cases shows no difference in the operation between these and the 80 per cent of flies which do not show these unusual effects. The Malpighian tubules are always considerably swollen and there appears to be an upset in the water regulating mechanism. This effect is found only in those flies which imbibe water and it is suggested that the operation may have stimulated some center, with the result that the flies imbibe more than they normally do.

The Effect of Severing the Recurrent Nerve

The question arises of whether the normal functioning of the ring gland is dependent upon its innervation. As shown by Day (1942) in Lucilia the recurrent nerve which supplies the ring gland is composed of fibers of three separate nerves. This compound nerve can easily be severed in the cervical region. This operation results in higher mortality than after extirpation of the ring gland. In one experiment, for example, 40 flies of both sexes of Sarcophaga were operated upon in this way, and of these only 16 survived for a period of one week. The effects of the operation are precisely similar to those following extirpation of the ring gland. The fat body cells enlarge, their nuclei become small and pycnotic, the oenocytes decrease in size and their cytoplasm becomes acidophilic, and the ovaries do not develop beyond Stage 1. The effect in Lucilia is shown in Figure 8 and that in Sarcophaga in Figure 10.

The histology of the corpus allatum after the severing of its nerve connections shows changes from the normal (Figure 3). The nerve endings are still conspicuous and little can be seen in the cytoplasm to suggest increased or decreased hormone output. However, striking changes occur in the cell size and in the nuclear size (Figure 3). Nuclear size is more easily measured than cell size. The nuclei of the operated flies measure from 12 to 14 microns in diameter, an increase of about 70 per cent over the diameter of the nuclei of the controls. The nucleolus also increases in size.

The Effects of Transplanting Ring Glands

Since it appeared from the extirpation experiments that corpora allata were concerned in maturation of the ovaries and directly or indirectly in the changes undergone by the fat body, the ring glands of ten-day females were transplanted into the abdomens of three-day females. Striking changes were induced in the hosts, probably attributable to the transplanted glands. The adult fat body of the host was markedly depleted, but the larval fat body cells still present showed a most unusual appearance as though their reserves were being mobilized more suddenly than is normal. This effect was found in individuals fixed 48 hours after the implantation. In flies fixed one week after the operation no larval, and extremely little adult fat body tissue could be found in sections. These experiments confirm the suggestion that the corpus allatum is concerned in the maturation of certain tissues.

A more significantly experiment seemed to be the implantation of ring glands into flies from which the ring gland had been extirpated for one week. It is hardly to be expected that the effects of extirpation of the ring gland could be reversed to the normal condition, for it has been shown that the normal activity of the corpus allatum is exhibited only when the gland is normally innervated. Two cases were fixed 46 hours after implanting the new gland. One showed an abundance of fat body, apparently intermediate between that of the fly from which the ring gland had been extirpated, and a normal of this age, and reduced oenocytes, with strongly basophilic cytoplasm rather than acidophilic as in flies after extirpation of the ring gland. The other showed a fat body and oenocytes which were essentially normal (Figure 12).

In a later section it will be shown that castration causes cytological changes in the corpus allatum of Lucilia, though not of Sarcophaga. However, the following experiment suggests that the corpora allata of castrate female Sarcophaga are physiologically altered. Ring glands of flies castrated seven days previously were transplanted as in the experiments just reported. Two cases were fixed 46 hours after implanting and two after seven days. Significant differences could be observed between these and the former series, but there are still definite effects of the implanted glands (Figure 11). These effects are sufficiently striking to confirm the suggestion that the ring gland from the castrated female had very different effects on the host from that from a normal female. It was noticed that the corpora allata of these transplanted glands, when studied in serial sections at autopsy, showed a slight indication of hypertrophy in a manner similar to that discussed above in denervated corpora allata *in situ*. Detailed analysis must await further experiments, but the generalization is warranted that castration causes physiological changes in the corpus allatum of Sarcophaga.

The Effects of Castration on Corpora Allata and Other Tissues

A. The experiments of Thomsen (1940) in which she extirpated ovaries were performed primarily on Calliphora. The operation resulted in hypertrophy of the corpora allata. Full confirmation has been obtained in my experiments with female *Lucilia sericata*, in which corpora allata showed considerable hypertrophy. The increase in size of the cells is illustrated in Figure 2 when compared with Figure 1. It will be noted that the increase is solely in cell size and there is no increase in cell number. The cytoplasm exhibits no more signs of activity than in the unoperated animals. Thomsen could offer no suggestion of the means by which the hypertrophy was brought about. A comparison with cases of hypertrophy of these glands, for example in Ephestia moths (Schrader, 1938) or termite royalties (Pflugfelder, 1938), does not assist, and it seems likely that a different mechanism is involved in each of these examples. There is a change in nuclear size comparable to that found in Sarcophaga, which results from severing the recurrent nerve.

B. Thomsen did not report experiments with male flies. These can be castrated even more easily than can females, for the testes are not so completely tracheated as the ovaries. However, no hypertrophy of the cells of the corpora allata, or any other change could be observed in male Lucilia either in behavior or in histology. Most cases were fixed seven days after the operation, but in a few cases even after 14 days no change could be observed.

C. Similar experiments were performed on Sarcophaga. Early observations indicated no hypertrophy of corpora allata. More extensive and detailed operations on both sexes were performed and the results carefully checked in histological preparations to determine whether castration was complete. No hypertrophy of the corpora allata comparable to that in female Lucilia was found in either sex of Sarcophaga (Figures 4 and 5).

D. It was thought that the accessory glands might have an effect. In male Sarcophaga the accessory glands alone and the accessory glands together with the testes were successfully removed. No change in corpora allata cells was found in ten operations.

In an activity apparently so fundamental in the physiology of the insect it is surprising that two genera as closely related as Lucilia and Sarcophaga should give such divergent results. Further discussion of their differences will be found on p. 139.

Castration of female Sarcophaga has no visible effects on the female accessory glands, or indeed on the majority of tissues. About 50 per cent of nuclei of fat body cells do, however, show varying degrees of pycnosis. The cytoplasm appears normal and the oenocytes are fully rounded and typical for this fly (Figure 6). No histological evidence was found in Sarcophaga to compare with the decrease in fat reported for castrated Melanoplus by Pfeiffer (1941).

The Effects of Cutting and Replacing Ovaries

In Sarcophaga an attempt was made to gain some indication of the effects on the ovaries by completely removing them from their attachments, and replacing them in the haemocoele. The flies were fixed after a period of one week and studied histologically. The ovaries had regained new tracheal connections, and appeared normal in every respect, with stage I oöcytes, as would be expected in flies fed only sugar. Fat body and oenocytes were normal and no effect of the operation was observed on the corpora allata.

General Discussion

We may assume for purposes of comparison with other insects that the effects of extirpation of the ring gland can be compared with allatectomy and cardiacectomy. The only report of cardiacectomy is to be found in a note by Pfeiffer (1939, p. 452–453) stating that "delay in molting has been consistently obtained by removing the corpora cardiaca" of Melanoplus. Allatectomy has been performed by Wigglesworth (1936) on Rhodnius where it was shown to result in loss of the ability to produce mature eggs. Degeneration occurred not only in the oöcytes but also in the follicular epithelium. Weed (1936) confirmed these results with Melanoplus. However, in Dixippus (Pflugfelder, 1937) allatectomy does not result in the loss of the ability to produce mature eggs though the fact that the corpora allata have some effect on the ovaries is shown by subsequent work (Pflugfelder, 1940). Subsequently Pflugfelder has reported a variety of effects from the removal of the corpora allata of Dixippus: the pericardial glands and ventral glands undergo considerable hypertrophy, and there are effects on the regeneration of lost limbs (1938b). These results lead to the conclusion that the corpora allata exert some influence on metabolism, but as Scharrer (1941) says "the question is how far this concept may explain all the special effects attributable to the glands."

From the present experiments there is evidence that the ring gland produces more than a single substance. As has been pointed out they indicate strongly that one of the primary effects of the ring gland is on the regulation of normal maturation. This is also true in the larva, as shown by the experiments of Burtt (1938) on Calliphora and of Hadorn and Neel (1938) on lgl Drosophila larva. Histological examination of "permanent larvae" of *Lucilia sericata* inhibited from pupating by removal of the ring gland shows a fat body which is unlike anything seen in normal larvae. The cells contain a large number of small acidophilic droplets, and a few larger droplets which stain with aniline blue. However, there is no regression or deterioration of the fat body cells as found in the adult fly, and a somewhat comparable picture is seen in a prepupa kept for one month in dry sand (see Mellanby, 1938). In such fat body cells many of the acidophilic droplets are considerably larger, are less regular in size, and are aggregated around the nucleus.

It is not known whether the hormone which permits normal pupation and whose removal results in fat body cells of this type is the same as that which plays a role in the removal of larval fat body in the adult fly. It seems unlikely that this is the case. And there is sufficient change in the ring gland that it is not necessary to assume that a single hormone is involved. A few experiments have been performed of transplanting glands from larvae to adults. Though they yielded no significant information, the conclusion that the ring gland produces a substance concerned with normal maturation seems incontravertible.

It has been shown that the effects produced by implanting a ring gland into a fly from which the ring gland had been extirpated are qualitatively different from those which result from severing the innervation of a gland *in situ*. It is possible that the effects may be produced by different concentrations of a single secretion but it appears likely that the substance causing the breakdown of larval tissues is not the same as that which affects the growth of the ovaries, the cytoplasm of the fat body, and the structure of the oenocytes (see also Vogt, 1940). As has been suggested by Pflugfelder the corpus allatum seems to have some influence on the metabolism of the insect. The obvious effects of the corpora allata of various insects on the ovaries have led to the suggestion that they may produce a hormone acting directly on the sex organs, at least in the female. The outstanding result of the experiments reported in this paper is that many tissues are affected by the corpus allatum. There is thus no reason to suppose that a sex hormone is produced by the corpus allatum. In fact, it seems more plausible to assume that the primary effect is on some general metabolic function. It is well known that many flies are unable to mature their eggs without a protein meal, while mature sperm are formed irrespective of the meal obtained by the male. Spermatogenesis is in no way affected by extirpation of the ring gland, while eggs, if formed, begin to undergo regression if the ring gland is removed even though protein be fed to the flies.

The results of castration are difficult to interpret. In female Lucilia, the only cells markedly affected are those of the corpora allata. In male Lucilia and in both sexes of Sarcophaga, castration has little effect either on the behaviour of the flies or on the cytological appearance of their tissues (see Figure 6). In the female Lucilia the corpus allatum hypertrophies after castration, and in this fly the oenocytes of the female are much smaller than those of the male. In Sarcophaga, castration does not result in any cytologically visible change in the corpus allatum of either sex, and in this fly the oenocytes of the female are almost indistinguishable from those of the male. This suggested that changes in the oenocytes might be induced by castration of female Lucilia and of male Sarcophaga, but none were found. However, this finding does not invalidate the general conclusion that the effect of the ring gland is probably primarily on some general metabolic process, perhaps acting through the oenocytes rather than directly on the ovary.

Summary

1. Evidence from extirpation and transplantation experiments suggests that the ring gland of *Lucilia sericata* and *Sarcophaga securifera* produces a hormone concerned with normal development. Its action can be seen in the larva where it results in puparium formation, and in the adult fly first in the changes which occur during the breakdown of the larval fat body cells and subsequently in the changes undergone by the adult fat body cells, the oenocytes, and the development of the ovaries.

2. These last two activities may be under the influence of a hormone (probably different from that influencing development), whose action seems to be on the general metabolic activity of the fly. The oenocytes undergo marked changes after extirpation of the ring gland. If these are concerned with some general metabolic function, as seems likely, the action may be primarily on them and the effects on fat body cells may be altered by implanting a ring gland into the abdomen of a fly, after extirpation of the ring gland, but this has no visible effect on oenocytes or on ovarian development.

3. Castration of adult female *Lucilia sericata* results in hypertrophy of the cells of the corpus allatum. No effect is produced in the male *Lucilia sericata* or in either sex in *Sarcophaga securifera*.

4. Destruction of the innervation of the ring gland of *Sarcophaga securifera* results in slight hypertrophy of the corpus allatum cells, and of their nuclei. The physiological significance of this hypertrophy is not yet known.

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